BODY FLUID DISTRIBUTION IN ACUTE HYPERCAPNIA

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SUMMARY PAGE

THE PROBLEM

To determine the effects of short exposure to CO_2 on body fluid distribution in laboratory animals.

FINDINGS

Guinea pigs and rats exposed to 2, 7, and 11% CO_2 for one hour showed no change in total body water, intracellular space or extracellular space. The volume of extracellular space in guinea pigs is 5% greater than extracellular space in rats when expressed as % total body water.

APPLICATION

These results are of interest to researchers and physicians involved in closed environment studies.

ADMINISTRATIVE INFORMATION

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ABSTRACT

Guinea pigs and rats were exposed to 1.9, 6.9 and 10.8% CO_2 for a period of one hour. Measurements of total body water (TBW), extracellular fluid (ECF) and intracellular fluid (ICF) were made using tritiated water and radioactive chloride (^{36}Cl). Control values for TBW, ECF, and ICF were 64.6, 28.5 and 36.1 for rats and 61.7, 30.6 and 31.1 for guinea pigs. These values did not change significantly under hypercapnia. These results indicate that although no measurable changes occurred in body fluid distribution under hypercapnia, the differences in body water distribution between guinea pigs and rates may help explain species differences in response to hypercapnic stress.

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BODY FLUID DISTRIBUTION IN ACUTE HYPERCAPNIA

INTRODUCTION

Recently, Steiner and Held[1] reported a rise of the apparent distribution volume of $^{35}SO_4$ = in a dog during acute respiratory acidosis. Malorny[2] has reported watershifts in red cells, liver and muscle tissue during acute exposure of rabbits, dogs and cats to 2-7% CO_2 for periods from 2-6 hours. Schaefer[3] has observed similar changes in the water content of some tissues of rats and guinea pigs after chronic exposure to 15% carbon dioxide. These observations indicate that body fluid distribution is altered in response to hypercapnia. In the present report, body fluid distribution in rats and guinea pigs under the influence of acute hypercapnia is studied.

MATERIALS AND METHODS

Male albino rats (Charles River Laboratories) and male albino guinea pigs (Charles River Laboratories) were used in all experiments. Weight ranges for animals were 260-410 grams and 250-400 grams for rats and guinea pigs, respectively. Animals are injected intraperitoneally with 0.5 ml of saline solution containing approximately 10μCi ^3H_2O (New England Nuclear) and 2μCi $Na^{36}Cl$ (Amersham Searle Corporation) and placed in metabolic cages in a 237 liter plexiglass box. One hour after injection time, the plexiglass box was closed and test gas passed through it at a rate of 8 liters/min. Test gases were air; 2.98% CO_2/20.73% O_2/balance N_2; 10.07% CO_2/20.72% O_2/balance N_2, or 14.98% CO_2/20.23% O_2/balance N_2. Fifty-five minutes after the box was closed, it was opened and the animals injected intraperitoneally with Pentabarbital Sodium (Sedasol; Evsco Pharmaceutical Corp.) at a dose of 0.1 gm/100 gm body weight or sufficient to anesthetize. The box was then closed until the anesthetic took effect.

Animals were removed, weighed and cut open with a midline abdominal incision. Blood was drawn from the abdominal aorta into a lightly heparinized syringe. Urine was collected from the bladder by by needle and syringe and combined with the urine collected in the metabolic cage and the volume recorded.

Counting of Samples

Blood in the case of rats, and blood and urine of guinea pigs was spun down at 7000 rpm for 10 minutes. Plasma and urine (0.1 ml) were placed in scintillation vials and 10.0 ml of scintillation fluid added. The scintillation fluid consisted of 0.25 grams/liter DMPOPOP, 7.00 grams/L PPO in toluene. Aqueous interfacing was accomplished by the addition of 10 vol BBS-3 (Beckman Instrument Co.) solubilizer to the scintillation fluid. Samples were counted for 100 minutes or 0.5% error in a Beckman LS 150 (Beckman Instrument Company) room temperature liquid scintillation system. Counting took place in three channels: external standard channel, tritium channel, and a channel which was cali-

brated to count approximately 80.0% of chloride disintegrations but 1% or less of tritium disintegrations. A background (bkgd) was prepared by adding 0.1 ml of saline to 10 ml of scintillation fluid.

Preparation of Standards

Quenching curves were prepared by counting tritium and chloride quenched standards (ICN Corporation) and plotting efficiency of counting against the external standard ratio. Activity of the 0.5 ml injected dose was determined by injecting an equivalent amount of labeled saline into a scintillation vial, diluting to 5 ml and counting duplicate 0.1 ml samples of this mixture.

Calculations

Counting efficiencies (eff.) for chloride^{36} in the chloride^{36} channel, chloride^{36} in the tritium channel and tritium in the tritium channel were determined from the quenching curves. Chloride^{36} dpm's were calculated by subtracting background cpm from chloride^{36} cpm and dividing by the efficiency of counting of chloride^{36} in the chloride^{36} channel (equation 1). Chloride^{36} overlap in the tritium channel

Equation 1.

$$\text{dpm}^{36}\text{Cl} = \frac{\text{cpm } ^{36}\text{Cl channel} - \text{bkdg}}{\text{eff. } ^{36}\text{Cl in } ^{36}\text{Cl channel}}$$

was determined by multiplying dpm of chloride^{36} by chloride^{36} counting efficiency in the tritium channel. Tritium cpm in the tritium channel were the total cpm in the tritium channel minus background and chloride^{36} overlap cpm. Tritium cpm were then divided by the counting efficiency of tritium in the tritium channel to give tritium dpms (equation 2).

Equation 2.

$$\text{dpm}^{3}\text{H} = \frac{\text{cpm}^{3}\text{H channel} - [(\text{dpm}^{36}\text{Cl} \times \text{eff. } ^{36}\text{Cl in } ^{3}\text{H channel}) + \text{bkgd}]}{\text{eff. } ^{3}\text{H in } ^{3}\text{H channel}}$$

By this method, the dpm of ^{36}Cl and ^{3}H were corrected by subtracting the dpm lost in the urine. The corrected injection dose (^{3}H or ^{36}Cl) was then divided by the plasma concentration (^{3}H or ^{36}Cl) to give the distribution volume.

The tritium distribution volume was corrected for plasma water content by multiplying by the decimal fraction of water in plasma. Values used were .954 for guinea pigs and .946 for the rats[3]. The chloride distribution volumes were corrected after the method of Swan, et al[4] by multiplying by (0.954) (0.95) for the guinea pigs and (0.946) (0.95) for the rats were 0.95 in the Gibbs-Donnan distribution ratio for chloride.

The corrected tritium distribution volume was taken to be total body water (TBW), the corrected ^{36}Cl distribution volume was extracellular fluid (ECF) and the difference was taken to be intracellular fluid (ICF).

RESULTS

Figure 1 shows that CO_2 equilibration profile over one hour for the flow of 8 liters/min thru the 237 liter box. It can be seen that equilibration did not occur in any exposure, but the profiles were

distinctly different by 15 minutes--that is, 10 and 15% CO_2 mixtures, respectively. The mean CO_2 concentrations calculated in intervals of five minutes were: 1.94, 6.95 and 10.80% CO_2 for the 2.98, 10.07, and 14.98% CO_2 mixtures, respectively.

Figures 2 and 3 and Table I give the body fluid distribution and standard deviations for rats and guinea pigs exposed to the three different CO_2 mixtures and air. For rats (figure 2), the control TBW was 64.6±1.3 and ECF was 28.5±0.8. These values did not change significantly under the conditions of this experiment.

For guinea pigs, control TBW and ECF were 61.7±0.6 and 30.6±0.5 body weight, respectively, and did not change significantly during the course of the exposures. There was a tendency for TBW to be elevated under the increased CO_2 levels.

Intracellular fluid (ICF) was taken to be the difference between TBW and ECF: Control ICF was 36.1 and 31.1 for rats and guinea pigs, respectively, and it did not change in any animal over the course of the experiment. In each condition when expressed as percent total body water, ECF and ICF for guinea pigs was statistically different from rats.

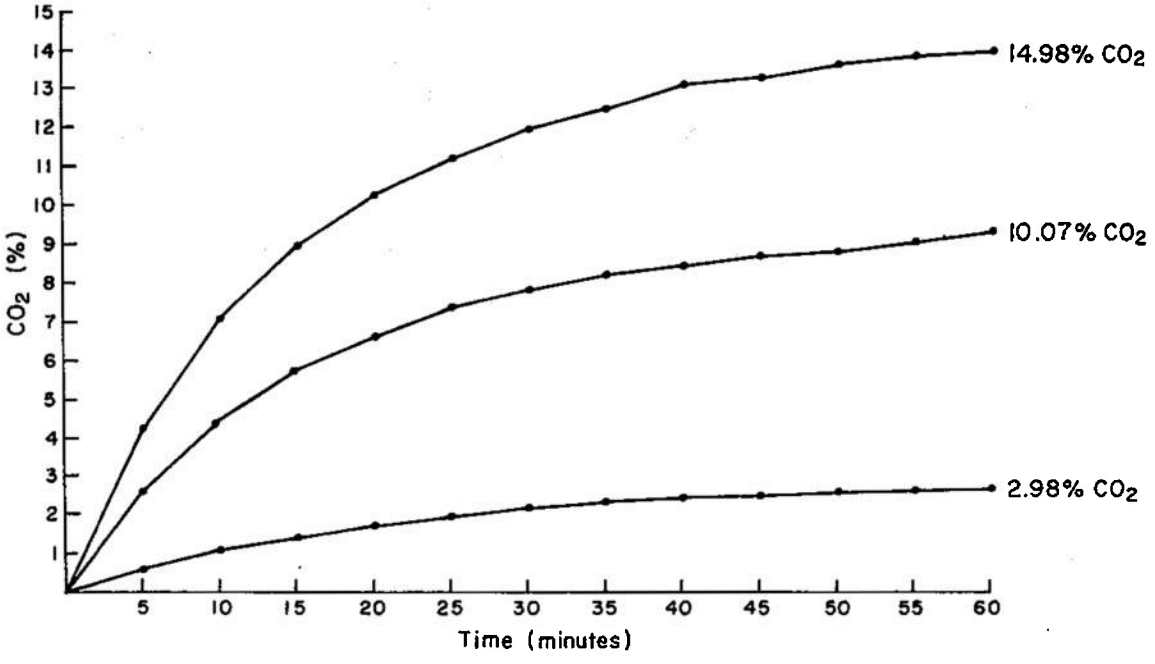

Fig. 1. Equilibration profiles for CO_2 in box used to expose rats and guinea pigs to hypercapnic medium

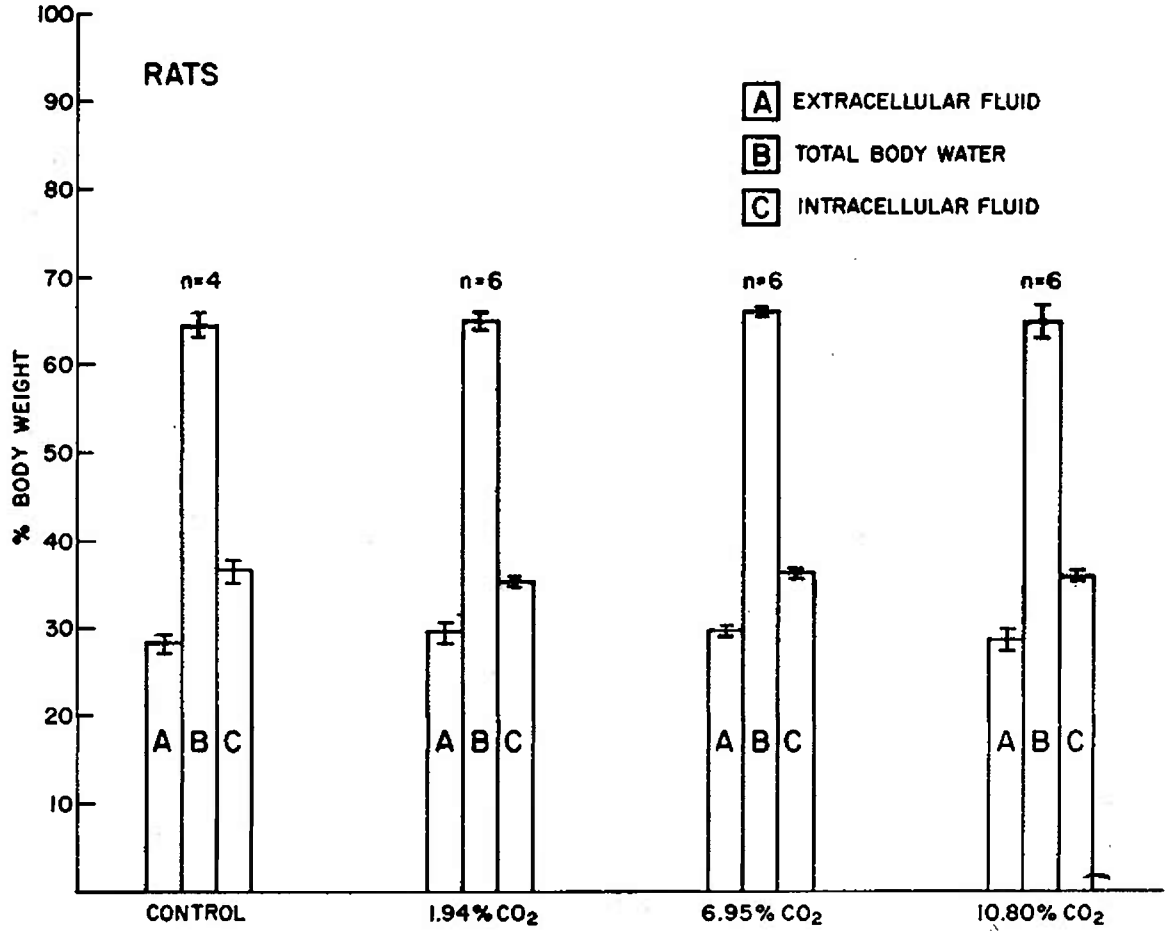

Fig. 2. Mean TBW, ECF and ICF (± 1 S.D.) expressed as % body weight for control and CO_2 exposed rats.

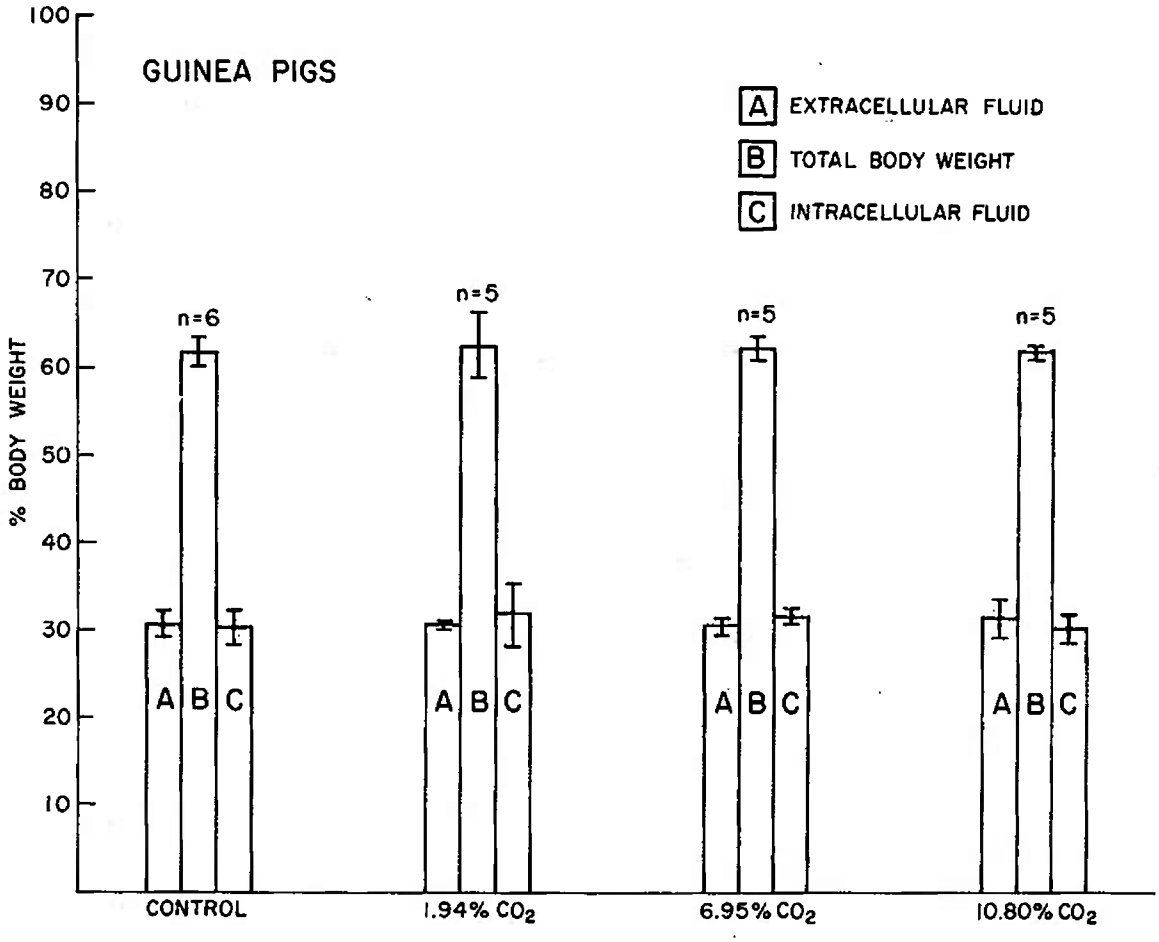

Fig. 3. Mean TBW, ECF and ICF (± 1 S.D.) expressed as % body weight for control and CO_2 exposed guinea pigs

DISCUSSION

The tritium method for total body water determination is assumed to overestimate the direct desiccation method by about 10%.[6] In our hands, however, the presently reported dual-labeling technique gives results similar to those found by desiccation in rats[6,7] and lower than those found by Foy[8] using tritium dilution. Values for TBW in guinea pigs are well below those of other investigators[7,8]. Before correcting for plasma water, our values are comparable to those found by Flexner[9] using deuterium oxide and Pace[10] using desiccation.

Our values for ECF are slightly lower than normally reported for chloride determinations in rats.[10] As far as we are able to determine, chloride spaces are not usually reported for guinea pigs.

The dual-labeling technique reported in the present study failed to detect a

significant change in body fluid content or distribution under the conditions used. The total body water of exposed guinea pigs and rats were slightly higher than control animals under all CO_2 concentrations; however, there was no observable urine retention in the exposed animals. Since no food or water was available to the animals during the course of the short exposure, the higher TBW's could reflect only a difference in hydration at the beginning of the experiment and not a CO_2 related effect.

Because of the possibility of differences in the hydration state causing erroneous differences in ECF volumes, ECF and ICF were also expressed as percent TBW (Table I). Again there were no significant changes noted with exposure to CO_2.

Acidosis could be expected to show an increase in the ECF as measured by $^{36}Cl^-$ due to the "chloride shift" into red blood cells in place of bicarbonate. An alteration in the distribution of $^{36}Cl^-$ extravascularly would also be expected

TABLE I

ECF AND ICF EXPRESSED AS %TBW FOR CONTROL AND CO_2 EXPOSED GUINEA PIGS AND RATS

		n	EXTRACELLULAR FLUID (ECF) (% TBW)±SEM	INTRACELLULAR FLUID (ICF) (% TBW)±SEM
Control	Rats	4	44.17±0.41	55.83±0.41
	Guinea Pigs	6	49.55±0.77	50.45±0.77
1.94% CO_2	Rats	6	45.07±0.27	54.93±0.27
	Guinea Pigs	5	49.23±1.28	50.77±1.28
6.95% CO_2	Rats	6	44.83±0.31	55.17±0.31
	Guinea Pigs	5	49.18±0.52	50.82±0.52
10.80% CO_2	Rats	6	44.39±0.24	55.61±0.24
	Guinea Pigs	5	51.12±0.93	48.88±0.93

to change the apparent ECF. That no change was noted in either rats or guinea pigs suggests that either no change occurred or that the technique was not sensitive enough to detect one.

Guinea pigs and rats show large species differences in their tolerance to CO_2 which is probably related to their difference in buffer capacity[12]. Recent studies demonstrated that the slope of CO_2 tritiation curves obtained in acute hypercapnia in guinea pigs is significantly lower than those of rats[13]. The difference between the two species is similar to the difference between the in vivo and in vitro CO_2 blood curves. The lower in vivo CO_2 buffer curve has been explained with a distribution of bicarbonate in blood and extracellular space[14]. The larger extracellular space (5%) found in guinea pigs could explain at least partially the lower CO_2 buffer curves in guinea pigs.

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13. ABSTRACT

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